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Confirmatory assays for detection of *Neisseria gonorrhoeae* using *porA* pseudogene Real-Time PCR based methods

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Background: Since the advent of molecular techniques, diagnosis of *Neisseria gonorrhoeae* has been ruin by false positive results when compared with culture, which is currently the gold standard. False positive results are often due to the cross-reaction of nucleic acid amplification test (NAAT) with closely related non-pathogenic *Neisseria* species. Regardless of the availability of commercial NAATs for *N. gonorrhoeae*, issues surrounding the specificity of these platforms persist.

Objectives: This research aims to institute, heighten and compare the sensitivity and specificity of previously available *N. gonorrhoeae* real-time assays which target the *porA* pseudogene.

Methods: In the course of the investigation, 156 *gonococci* specimens and 30 non-*gonococci* culture specimens were used. Optimization of the *PorA* pseudogene real-time PCR was carried out by varying the concentration of magnesium chloride as follows: 5mM ranges between 19.08 (4.31) and 23.27 (17.57), 4mM ranges from 17.18 (1.15) and 22.01 (16.43) and for 3mM the range is from 21.71 (2.20) and 27.33 (15.27) with the standard deviation in bracket and as well as the forward and reverse primers which have varying concentration as 50mM, 300mM and 900mM for both.

Results: The results obtained show the high specificity of the assays for all 156 *gonococci* culture specimens gave positive results, whilst the 30 non-*gonococci* specimens gave negative results. This shows that *PorA* pseudogene real-time PCR is a suitable assay for the confirmation of putative *N. gonorrhoeae* cultures and can assist in identification, particularly in cases where traditional biochemical and immunology tests have failed. The potential of the *PorA* pseudogene real-time PCR to detect the presence of *N. gonorrhoeae* specific DNA directly from clinical samples was then evaluated. An initial experiment was performed which involved the addition of a primer and probe set which acted as an internal control, it was determined that the internal control did not compromise the sensitivity of the *PorA* pseudogene real-time PCR assay and could be used reliably to screen for assay inhibition. The *PorA* pseudogene real-time PCR was then used to examine some clinical specimens which had been examined previously at three laboratories, each of which different commercial *N. gonorrhoeae* NAAT platforms were used. The results from this investigation show a high specificity evidence of *PorA* pseudogene real-time PCR when compared to previous results obtained from the other laboratories.

Conclusion: The study has succeeded in establishing to very large extent that the *PorA* pseudogene real-time PCR is a very valuable assay for the detection and confirmation of *N. gonorrhoeae* specific DNA from both putative cultures and directly from clinical samples.

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